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Manual

AMR-ve Genotyping Kit

Array Hybridisation Kit for DNA-based detection of the most common resistance genes of Gram-negative bacteria

Kit order number: 205300050

50 reactions (ArrayTube format)

For Research Use Only. Not for Use in Diagnostic Procedures.

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BACKGROUND

The AMR-ve Genotyping Kit allows a quick and simple method for the detection of the most common resistance genes of Gram-negative organisms, using an ArrayTube™ based assay.

RNA-free, un-fragmented genomic DNA from pure and monoclonal Gram-negative bacteria (e.g. *Escherichia coli* or *Salmonella enterica*) is amplified approximately 45-fold and internally labelled with biotin-11-dUTP using a linear amplification protocol. In contrast to standard PCR, a multiplex primer extension reaction is performed with two nested primers per target in each cycle. Two-versus-one primer for each target increase and synchronize the yield of biotin labelled single stranded (ss) DNA product for all markers. This allows a simultaneous sequence specific labelling and amplification of an essentially unlimited number of targets. However, sensitivity is lower than in a standard PCR (whereas contamination with amplicons is nearly impossible) and for that reason the method is restricted to clonal colony material and cannot be performed on samples such as swabs or other patient samples. Resulting biotin labelled ssDNA is transferred and hybridised to DNA oligonucleotide microarrays with 89 probes for different genetic markers plus controls. All of them are spotted in duplicates.

The target set consists of a variety of antibiotic resistance genes of different Gram-negative bacteria, family-specific markers for *Enterobacteriacea*e and different genus markers for *Salmonella, Escherichia* and *Shigella*. The array contains markers for genes associated with:

- **family identification**
- **streptogramin A/B resistance**
- **aminoaminoglycoside resistance**
- **beta lactam resistance**
- **chloramphenicol resistance**
- **macrolide resistance**
- **quinolone resistance**
- **sulphonamide resistance**
- **tetracycline resistance**
- **trimethoprim resistance**
- **integrases**

GENERAL INSTRUCTIONS FOR USE

Intended Use

For Research Use Only. Not for Use in Diagnostic Procedures.

This kit allows the detection of resistance-associated genes in isolates of Gram-negative bacteria/*Enterobacteriaceae* for research and epidemiological applications. It must not be used as a substitute for phenotypic susceptibility tests and for the guidance of antibiotic therapy.

Please note: It cannot be used for Gram-positives.

Specifications

Upon receipt, the assay components need to be stored at different temperatures as specified on the package insert. The assay is to be performed at an ambient temperature of 18°C to 28°C.

Technical Support

Email: cct.home@clondiag.com

Phone: +49 (0) 36 41 3111-155

Fax: + 49 (0) 36 41 3111-120

For up-to-date information regarding the kit, please visit our website at

http://alere-technologies.com/en/products/lab-solutions/amr-ve-genotyping.html

Safety Precautions

- **The kit is intended for use by personnel that are trained in microbiological and molecular methods. Preparation of DNA from bacterial colonies (clones) requires expertise in microbiology and the local regulations for handling of pathogenic microorganisms (usually biosafety level 2) are to be obeyed.**
- **Isolated, cell-free DNA may be processed without further biosafety precautions, although bacterial contaminations need to be ruled out.**
- **Always wear protective clothes as required for laboratory work according to your local regulations.**

Material Safety Data Sheets (MSDS)

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC: 1005, 1008(1999)] and the latest regulations (EC) 1272/2008 (CLP) and 1907/2006 (REACH), the enclosed reagents do not require a Material Safety Data Sheet (MSDS), **except Hybridisation Buffer C1. The MSDS can be downloaded via our website from any lab solutions product page (e.g. http://alere-technologies.com/en/products/lab-solutions.html).** All other reagents do not contain more than 1 % of a component classified as hazardous and do not contain more than 0.1 % of a component classified as carcinogenic. Nevertheless, the buffers may cause irritation if they come into contact with eyes or skin, and may cause harm if swallowed. The regular precautions associated with laboratory work should be obeyed (e.g., wear protective goggles, gloves and lab coat and avoid contact with the reagents). If liquid is spilled, clean with a disinfectant and/or laboratory detergent and water.

Alere assumes no liability for damage resulting from handling or contact with these products. If you have any questions please contact our Technical Support (see above).

Shipping Precautions

RID/ADR: “Kein Gefahrgut”/ No dangerous goods

IMDG: No dangerous goods

IATA: No dangerous goods

DEVICES, SOFTWARE AND REAGENTS

Devices

- ArrayMate Reader (to be ordered separately, for details see below)
- Alternatively, Reader ATR03 (to be ordered separately, for details see below)
- Iconoclust software (provided with the reader)
- Report Generator (optional)

Whilst the **AMR-ve Genotyping** assay runs both on the ArrayMate Reader and on the ATR03 reader, respectively, this manual describes the reading of processed AT on the ArrayMate reader only. If you want to use ATR03, please refer to the latest version of the ATR03 manual or contact us.

Assay specific software (plug-in) is delivered with the reader or can be downloaded from our website, where it will occasionally be updated.

The ArrayMate Reader by default has all software on board. However, the **AMR-ve Genotyping** assay specific package might be missing, e.g., if you obtained the device for the use with another assay. Then you may need to install it separately. It will be provided upon kit order, and can also be downloaded from our website as discussed above. No issues regarding compatibility of software have been observed with the ArrayMate device.

The ATR03 reader requires several pieces of software to be installed on an external PC (please refer to the latest version of the ATR03 manual for details). The **AMR-ve Genotyping** assay specific software is not compatible with iconoclust versions older than version 4.4.

Software Installation

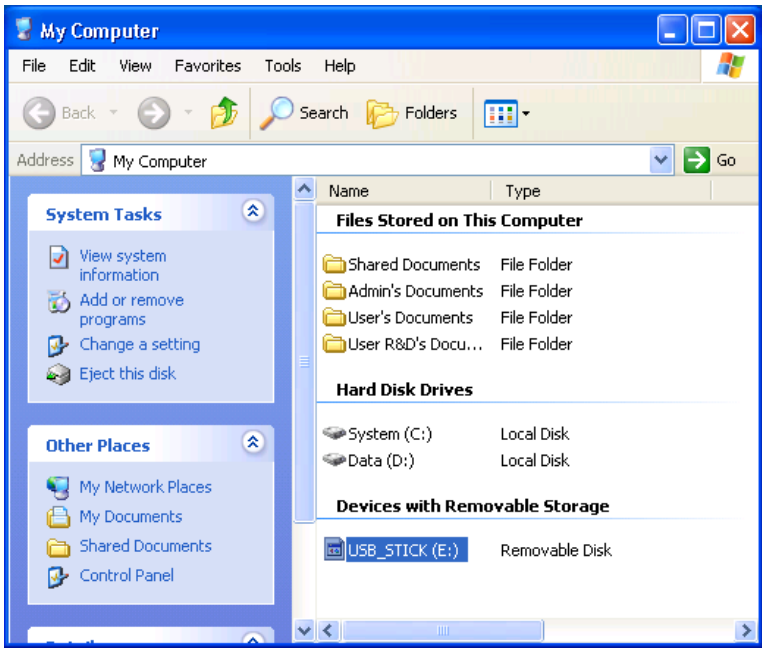
For analysis of the final image of the DNA microarray on the ArrayMate a specific software plug-in is required. This software plugin can be downloaded from our website www.alere-technologies.com under **Downloads** (plug-ins). Please install it on your reader according to the following instructions.

Assay Plugin and SDK for the ArrayMate

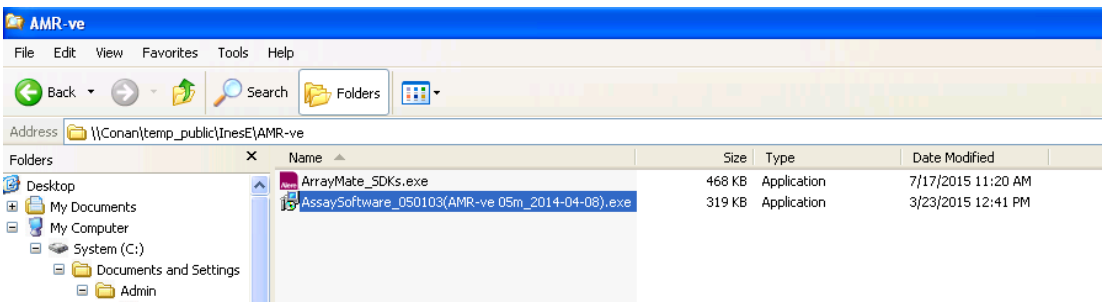
The following instruction describes the installation of the AssayPlugin and ArrayMate installation software (SDK).

Download the AssayPlugin **and** the ArrayMate SDK from http://alere-technologies.com/en/products/lab-solutions/amr-ve-genotyping.html

1. Copy downloaded files (Plugin and SDK setup) to an USB Memory Stick and connect it to the ArrayMate.
2. Log on as user ***admin*** to the ArrayMate (default password: 12345).
3. Open the **Windows Explorer** and navigate to the downloaded **setup files.**



4. Start installation: double click on setup file of the AssayPlugin.



5. The welcome screen of the setup appears.

Setup - ArrayMateAssay_R_D "050103 AMR-ve 05m"
Welcome to the ArrayMateAssay_R_D "050103 AMR-ve 05m" Setup Wizard
This will install ArrayMateAssay_R_D 050103 (AMR-ve 05m 2014-04-08) on your computer.
It is recommended that you close all other applications before continuing.
Click Next to continue, or Cancel to exit Setup.
Alere
Next >
Cancel

6. Follow the instructions and press **Finish** to complete the installation.
7. Repeat this process for the **SDK** Setup.
8. Log off and log in again as **User R&D** (default password: abcde).

Test the AssayPlugin

The Software installation can be tested with the unprocessed Array by the following steps:

1. Log on to the ArrayMate in **User R&D** Mode (default password: abcde) and start a **New Run.**

2. Choose automatic detection in **Experiment Infos** and press **Next**. Place the ArrayTube rack with an unprocessed ArrayTube into the ArrayMate than press **Next.**

   After the Experiment Run the ArrayMate automatically enters the Archive mode and displays the results of the last experiment.

3. Each cell of the columns **image**, **raw data** and **results** must contain an “X”. Otherwise, please retry the installation process of the AssayPlugin and the installation software (SDK).

expe...	sample ID	position	assay	image	raw data	results
01-A	-	1	050103	X	X	X
01-B	-	2	050103	X	X	X
01-C	-	3	050103	X	X	X
01-D	-	4	050103	X	X	X
01-E	-	5	050103	X	X	X
01-F	-	6	050103	X	X	X

Kit Components, Storage and Stability

All reagents are provided in surplus (see below). If necessary, all components may be ordered separately; please refer to the catalogue reference numbers (Cat#) at the end of this manual. For pricing please contact your local representative or our customer service, respectively.

The expiry date can be found on each bottle and on the outer packaging. All components have been stability tested for short term shipment (<1 week) at ambient temperature (< 37°C). The assay components with limited stability are D1 and C3. The other kit components have proven to be stable six months post expiry.

DNA Labelling and Amplification

- **B1**$^{\text{AMR-ve}}$: Labelling Buffer, Store at 2°C to 8°C. Surplus: 45 %.
- **B2**: Labelling Enzyme, Store at 2°C to 8°C. Surplus: 300 %.

Hybridisation and Detection

- ArrayTubes (10 x 5 samples), protected against light and sealed under inert gas. Store at 18°C to 28°C. After opening, tubes are to be used within two weeks. Close unused ArrayTubes, protect them against humidity and dust and store in the dark. Avoid ANY touching or scratching the microarray on the bottom of the vial.

Please note: *Do not store or handle unused wells above 60 % relative humidity since this may irreversibly corrode the spots.*

- **C1**: Hybridisation Buffer. Store at 18°C to 28°C. Protect against sunlight. Surplus: 100 %.
- **C2**: Washing Buffer 1. Store at 18°C to 28°C. Surplus: 140 %.

- **C3**: 100x HRP Conjugate. Store at 2°C to 8°C. Surplus: 300 %.
- **C4**: Conjugate Buffer. Store at 18°C to 28°C. Surplus: 500 %
- **C5**: Washing Buffer 2. Store at 18°C to 28°C. Surplus: 140 %.
- **D1**: Horseradish Peroxidase Substrate. Store at 2°C to 8°C. Protect against direct sunlight. Surplus: 200 %.
- **Optional: CM^{EDL}**: Reference DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2), c_{DNA} = 0.1-0.4 µg/µL. Store at 2°C to 8°C. Sufficient for 5-6 tests.

Components required but not provided

- Growth media for the cultivation of bacterial isolates. The test should be performed with colonies harvested from 2xTY. Other rich media (e.g. Columbia Blood Agar, Standard 1 or LB) also will suffice, but have not systematically been tested. Liquid media should not been used because contaminations or mixed cultures cannot be ruled out easily.
- Equipment and consumables needed for the cultivation of bacteria (incubator, inoculation loops, Petri dishes)
- DNA preparation kits:
  The assay has been tested with the DNeasy Blood & Tissue Kit from Qiagen (cat# 69504) and the DNA preparation kit for Qiagen´s EZ1 automated device (cat# 953034).
- Equipment needed for DNA isolation, e.g. pipettes, centrifuge, thermoshaker or automated device (see above)
- 1x PBS
- RNAse A (we recommend Qiagen's RNase A solution, 100 mg/ml, Qiagen Cat# 19101)
- Photometer (OD 260 nm) for measuring the concentration of DNA
- Equipment for non-denaturing agarose DNA gel electrophoresis for quality control of DNA
- Thermocycler for PCR
- Thermoshaker

  Please note: *We recommend Eppendorf's Thermomixer Comfort equipped with a heating block for 1.5ml tubes.*

- Pipettes: suitable for 1µL-5µL volumes, 90µL, 100µL, 200µL, 1000µL
- Multichannel Pipettes for 100-200 µL
- Sterile reaction vials suitable for PCR (VWR Cat# 732-0098)
- Ultrapure (PCR grade) water
- Pasteur pipettes (VWR Cat# 612-2856)

PROTOCOLS

Culturing and Harvesting Bacterial Cells

Enterobacteriacae are potential pathogens. All procedures for cultivation of the bacteria and DNA preparation need to be performed by properly trained staff in a biosafety level 2 facility.

Grow bacteria on suitable agar (see above) overnight at 37°C or 48 hrs at room temperature. Make sure that you have a pure, monoclonal culture. Contamination with other bacteria needs to be strictly avoided as this can introduce false positive signals and patterns.

Extraction of DNA

The required sample type for the **AMR-ve Genotyping** test is 0.5-2 µg (cDNA = 0.1-0.4 µg/µL) of intact DNA from **a single clone** of Gram-negative bacteria. This is much more DNA than for standard PCR applications (see Introduction).

The DNA specimen needs to be free of RNA and it should not be fragmented. This can be determined by agarose gel electrophoresis.

DNA should not be prepared by disrupting cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. Most performance problems are due to insufficient amounts or quality of DNA preparation. We therefore strongly recommend following the protocols outlined below.

The use of automated systems for DNA preparation (EZ1, Qiacube, Magnapure etc.) has not yet been systematically evaluated with this assay. While there are positive experiences with some of our other assays, we recommend testing some known strains for evaluation prior to routine

use of these systems. Lysis steps and addition of RNase should be performed as described below before loading the samples in an automated system for DNA preparation.

Extraction of DNA by Spin Columns (e.g. Qiagen DNeasy Blood&Tissue)

- Add an inoculating loop full of monoclonal colony material of the *E. coli* isolate to 0.2 ml 1xPBS and vortex thoroughly.

Loop empty Loop full

It is important to harvest enough bacteria; this is a prerequisite for extraction of a sufficient amount of DNA.

Take an inoculating loop of 1 mm diameter filled with bacteria as shown in the left picture.

- Proceed with the DNA preparation protocol of the DNA preparation kit. For the Qiagen DNeasy Blood&Tissue Kit that is as follows:
- Add 25 µL proteinase K (Qiagen Kit, or equivalent) and add 200 µL buffer AL (Qiagen Kit)
- Vortex shortly or shake vigorously.
- Incubate for 30-60 min at 56°C and 550 rpm in the thermoshaker.
- Add 4 µL RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing
- Add 200 µL ethanol (96-100%).
- Vortex the sample and centrifuge shortly.
- Transfer the complete content of the tube into a spin column that is placed in a 2 ml collection tube.
- Centrifuge at room temperature, time and speed need to be determined depending on viscosity of the sample and type of centrifuge used. All liquid should be collected in the collection tube afterwards.
- Discard collection tube with liquids.

- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µL Buffer AW1.
- Centrifuge at room temperature.
- Discard collection tube with liquids.
- Place the spin column in a new 2 ml collection tube (provided with the kit).
- Add 500 µL Buffer AW2.
- Centrifuge at room temperature, the membrane of the spin column should be dry, and all liquid should be in the collection tube.
- Discard collection tube with liquids.
- Place the spin column in a clean 1.5 ml tube (provided with the kit).
- Add 50 µL Buffer AE (or PCR grade distilled water) directly onto the membrane of the spin column.
- Incubate at room temperature for 1 min to elute DNA.
- Centrifuge.
- Optional: add another 50 µL Buffer AE (or PCR grade distilled water) directly onto the membrane, incubate at room temperature for 1 min and centrifuge again.
- Discard the spin column.

Please note: *Ethanol from Washing Buffers strongly inhibits the enzymes used in the assay. A contamination with washing Buffer might occur during elution of prepared DNA by drops adhering to the funnel of the spin columns. Thus these funnels should be gently touched and tried with sterile filter paper or wipes prior to the elution step. Alternatively, prepared DNA can shortly be heated to evaporate ethanol (e.g., 10 min at 70°C).*

- Check for DNA integrity and absence of RNA (e.g., agarose gel). If necessary, you might perform another digestion step with additional RNase A (not provided). Measure DNA

concentration (A_{260} method), it shouldn´t be less than 0.1 µg/µL. The concentration might be increased by heating and evaporation of water, or by using a speed vac centrifuge.

Extraction of DNA by Automated Device

The assay has been tested with Qiagen´s EZ1. Other systems also can be used. However, performance should be checked with some known reference strains prior to routine use.

- Add 10 µL proteinase K and add 100 µL buffer AL to harvested bacteria.
- Vortex shortly or shake vigorously.
- Incubate sample, 45-60 min at 56 °C and 550 rpm in the thermomixer.
- Add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing
- When the cells are lysed, proceed by performing the tissue lysis protocol (Bacteria card) for Qiagen´s EZ1
- *For Qiagen´s EZ1*: Front row: empty elution tubes (1.5 ml); Second row: tip holder with tips; Third row: empty; Back row: sample tube with conical tip (2 ml) with the 200 µL sample volume. Set tissue lysis protocol with a set sample volume of 200 µL and an elution volume of 50 µL.
- Concentrate DNA and evaporate traces of solvents by heating the sample, at 70 °C for 5-10 minutes.

Linear Amplification and Internal Biotin Labelling

Please keep in mind the limited surplus of reagents whilst pipetting. The surplus of B1^{AMR-ve} labelling reagent is 45%.

- Prepare a Master Mix by combining 4.9 µL of B1^{AMR-ve} labelling reagent and 0.1 µL of B2 (DNA polymerase) per sample.
- Add 5 µL of *E. coli* DNA (c_{DNA} = 0.1-0.4 µg/µL) prepared as described above to 5 µL of the Master Mix (B1^{AMR-ve}/B2). Do not forget to label the vial!
- Perform amplification in a pre-programmed thermocycler (such as Mastercycler gradient with heated lid) according to following protocol:

<table>
<tr><td colspan="2">Pre-heat cover/lid to 105°C</td></tr>
<tr><td>300 sec at 96°C</td><td></td></tr>
<tr><td rowspan="3">45 cycles with</td><td>20 sec at 50°C</td></tr>
<tr><td>30 sec at 72°C</td></tr>
<tr><td>20 sec at 96°C</td></tr>
<tr><td colspan="2">Cool down to 4°C, hold</td></tr>
</table>

- The amplification products can be stored frozen until usage.

Please note: When using another device, some adaptations might be necessary. Before starting routine use, please test the protocol with a few known reference strains and the control DNA (CM) supplied with the kit.

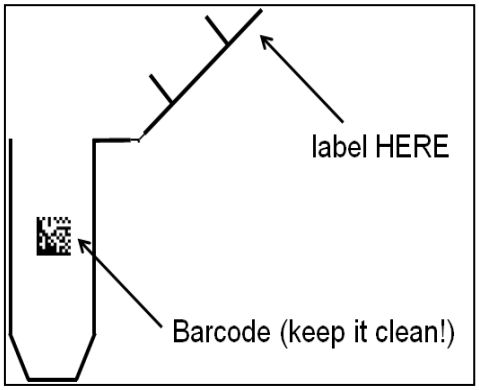
Hybridisation procedure

General Remarks - Handling of Arrays

- *Never touch the array surface!*

- *Avoid complete drying of the array surface during processing!*
- *Do not allow it to stay without liquid for more than two minutes!*
- *Never rinse the wells with distilled water after the hybridisation step, only use C2 Washing Buffer!*

Always label your ArrayTubes with a laboratory marker at the recommended position. Never label them on the bottom or across the data matrix barcode! This may cause an error.



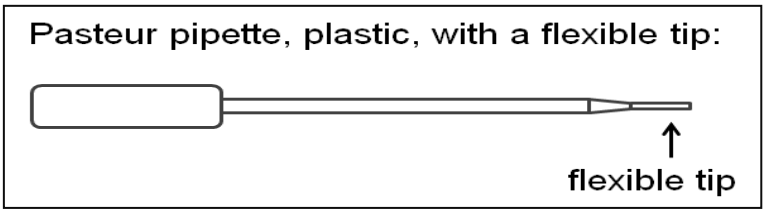
Avoid contact of data matrix barcode with organic solvents! The ArrayMate needs the information encoded in the data matrix to perform the assay.

Avoid touching the bottom of the microarray and keep it clean.

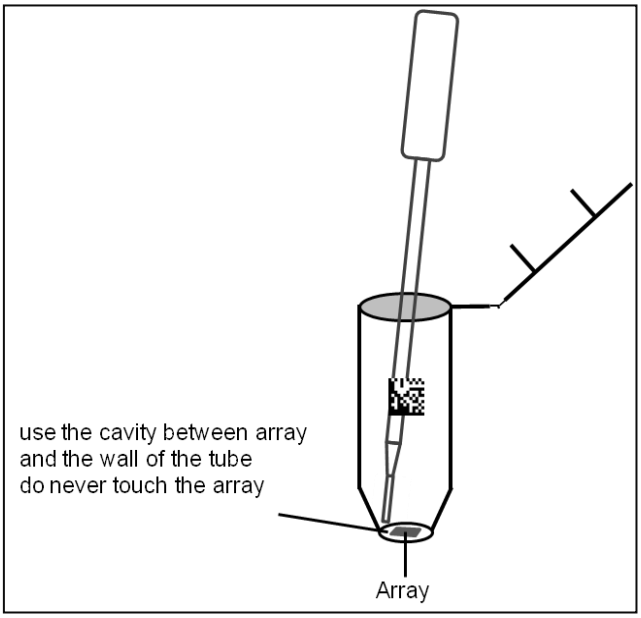
General Remarks - Handling of Liquids

We recommend the use of a multichannel pipette and reagent reservoirs. Please keep in mind the limited surplus of C1 (100%).

We strongly recommend that the liquid is removed by pipetting. Fine tipped soft, disposable Pasteur pipettes are suited best (such as VWR Cat# 612-2856).



Always place the pipette tip at the cavity between the array and the wall of the reagent well. If you touch the array surface, probes may be scratched off and this may cause an error.



General Remarks – the Substrate (Precipitating Dye) D1

An appropriate amount of substrate (precipitating dye) should be filled into an Eppendorf tube and taken out of the refrigerator when starting the procedure allowing it to pre-warm to room temperature/25°C. Cold D1 may yield weak signals. D1 should be centrifuged (1 min, 13,000 rpm) prior to use to remove bubbles as well as possible precipitates.

Triggered by peroxidase, in case of positive reactions, the dye precipitates but it is not covalently bound. The precipitate can be dissolved by vigorous shaking. Thus the arrays must not be shaken, dropped or moved abruptly during the staining procedure and afterwards.

After completion of staining, do **not** remove reagent D1 and scan immediately. The dye precipitate fades slowly in presence of liquids.

General Remarks - Thermoshakers

The correct temperature within the vessels is essential; therefore always use the appropriate equipment for heating. Because of a possibly inhomogeneous distribution of the temperature within the heating block and because of possible differences between displayed and actual

temperatures, the use of different brands of thermoshakers might affect test performance. We tested the assay with BioShake iQ by Quantifoil Instruments (http://www.qinstruments.com/) equipped with a customised heating block designed to fit ArrayTubes and Eppendorf's Thermomixer Comfort, equipped with a heating block for 1.5 ml Eppendorf tubes. Thus we recommend the use of either device. When using other devices, some modifications to the protocol might be necessary. Before starting routine use, please test the protocol with a few known reference strains or the control DNA supplied with the kit.

Protocol

Preparation of the hybridisation mixture

- Pre-heat the thermoshaker to 55 °C
- Add 90 µL of buffer C1 to each labelling product, mix gently (vigorous mixing results in foaming) and put aside.

Pre-washing of the arrays (2 washing steps)

- Remove the ArrayTube from the bag (open the bag at its predetermined breaking point)
- Add 500 µL of ultrapure water to each tube
- Incubate in the thermoshaker at 55 °C, 550 rpm for 2 minutes
- Remove and discard the water WITHOUT TOUCHING THE ARRAY SURFACE
- Add 500 µL buffer C1 to each tube
- Incubate in the thermoshaker at 55 °C, 550 rpm for 4 minutes
- Remove and discard buffer C1
- Proceed promptly (hybridisation mixtures must be ready when buffer C1 is removed)

Hybridisation

- Transfer each hybridisation mixture (100 µL) to a prepared ArrayTube (avoid extensive foaming)
- Incubate for one hour at 55°C and 550 rpm on a thermoshaker

Note: Meanwhile, login to the ArrayMate device and prepare your worklist (see section "worklist" S. 21)

Dilute Streptavidin-Horseradish-Peroxidase (C3, C4)

- Combine reagent C3 (Streptavidin-Horseradish-Peroxidase) and Buffer C4 in a ratio of 1:100, the mixture is stable for 1 day at room temperature; C3 is delivered with a surplus of 300 %, C4 is delivered with a surplus of 500 %.

Pipetting scheme: ArrayTubes (AT)

	1 AT	2-3 ATs	4-6 ATs	7-10 ATs	11-15 ATs
C3	1.5 µL	3.5 µL	7 µL	11 µL	16 µL
C4	150 µL	350 µL	700 µL	1100 µL	1600 µL

- Put aside at room temperature until use.

Pre-warm the staining reagent D1

- Transfer enough reagent D1 into a separate vessel (e.g. a clean and sterile centrifuge tube), 100 µL for each well and a surplus of not more than 20 %
- Put aside at 20 to 25°C until use.

Washing after hybridisation

- Please keep in mind the limited surplus of C2 (140 %).
- Remove the ArrayTubes from the thermoshaker.
- Set the thermoshaker to 30°C for the following steps.
- Carefully open the tubes and remove the hybridisation mixture as completely as possible (without touching the array surface).

- 1st Washing step after hybridisation: add 500 µL of buffer C2 and incubate in the thermoshaker at 30°C, 550 rpm for 5 min, remove and discard the washing solution.
- 2nd Washing step after hybridisation: repeat 1st Washing step.

Please note: A carryover of more than 1 % of buffer C1 to the next step will denature the HRP.

Addition of HRP-conjugate

Please note: Reagent C3 contains Streptavidin-Horseradish Peroxidase (HRP) that would denature and lose its activity at 55°C. Do NEVER incubate above 30°C. Make sure that the thermoshaker has cooled down before mounting the ArrayTubes! Please keep in mind the limited surplus of C3 (300 %).

- Add 100 µL of prepared C3/C4 mixture to each tube.
- Incubate in the thermoshaker at 30°C, 550 rpm for 10 minutes.
- Remove and discard C3/C4 mixture completely.

Washing step after binding of conjugate Addition of HRP-conjugate

- Please keep in mind the limited surplus of C5 (140 %).
- 1st Washing step after conjugation: add 500 µL of buffer C5, incubate in the thermoshaker at 30°C, 550 rpm for 1 minute, remove and discard the washing solution.
- 2nd Washing step after conjugation: Repeat 1st Washing step.

Please note: A carryover of more than 0.5 % of C3/C4 into the following staining reagent will create black particles which in the worst case may mimic signals (hybridised spots). On the same time, real signals may appear pale due to competition of soluble Horseradish Peroxidase with the DNA-bound enzyme for substrate molecules.

Staining of bound HRP-conjugate

Please note: *Do **not move** ArrayTubes during staining. The reagent D1 contains a substrate for Horseradish Peroxidase. Please keep in mind the limited surplus of D1 (200 %).*

- Set the thermoshaker to 30°C for the following steps.
- Add 100 µL pre-warmed reagent D1 to each well (supernatant of centrifuged D1 without precipitate).
- Incubate at 25°C **WITHOUT** agitation for 10 min.
- Read out **WITHOUT** removing D1 (removal of D1 would leave air bubbles within the tubes).

Please note: *The ArrayTubes as used in this kit do have a different geometry than the 8-well ArrayStrips that are used in other kits. Therefore, unlike the directive for ArrayStrips, D1 is NOT to be removed from the ArrayTubes before reading.*

Check immediately all images for cleanliness (i.e., absence of dust particles, residual liquids) and for good focus. Dust particles and residual fluids inside the vial can be removed by cautiously washing twice with 200 µL PCR-grade distilled water. If necessary, scan and process again.

Data Analysis

Starting the ArrayMate Reader

- We recommend starting the ArrayMate Reader after starting the hybridisation; this allows the convenience of starting the device and importing the worklist file (see below).
- Please note that this is a short instruction only. For more detailed information please refer to the ArrayMate User Manual.
- Switch on the ArrayMate (main switch on the rear below the electric cable plug, operating switch on the bottom/left corner of the front side).
- Switch on the screen (switch right hand below the screen).

- Log-in as ***R&D User*** (Research and Development User) for full access to test specific software (default password: abcde). If you log-in as **User**, you will obtain only raw values, but neither positives/negatives interpretation nor strain assignment. The **Administrator** log-in (default password: 12345) will allow the installation of a new assay specific plug-in, which can be downloaded at http://alere-technologies.com.
- The user interface will be loaded, ArrayMate performs internal testing. This will require slightly less than a minute.
- Click on the icon **New Run** (left upper edge of the screen). A suggestion for a run name / folder name for the new run appears in the top line of the screen). You may modify or change the experiment name at your convenience.

Worklist

A **Worklist** file allows an identifier, such as a laboratory or sample number, to be linked to the respective array position on the ArrayStrip. For privacy reasons, arrays should not be identified by patient names. Worklists can be generated using spreadsheet software such as EXCEL (see below), but must be saved in the *.txt file format that can be imported into the test-specific ArrayMate software. **Do not use special characters** (such as: ; ()[] / \ ä ü etc.).

- Create a list with at least three columns that have headers written in the first line. The following headers are obligatory (in this order): position / sampleID / assayID (Table 1).
- Positions are consecutively numbered from 1 to a maximum of 6. Do not leave empty lines in the worklist. If you use EXCEL, position numbers should be entered into column A.
- Sample IDs are strain/sample/laboratory numbers such as exported from your LIMS (or assigned in any different way). Patients' names should not be used as sample IDs.
- The Assay ID allows the system to identify the current test and to correctly use information on layout, spot number, and identity etc. The **AMR-ve Genotyping Kit** has the Assay ID: **050103**.

  Please note: When entering assay IDs manually, make sure to enter the correct number as entering wrong numbers could lead to errors or loss of data.

- We recommend using a printout of the worklist as a template for pipetting.
- Save the worklist as **tab separated *.txt** file on the memory stick provided together with the ArrayMate.
- To avoid confusion, make sure that worklists are named unambiguously or that worklists from earlier experiments are deleted.
- You may use the software tool **Worklist Generator** to create a worklist easily.

  http://alere-technologies.com/en/products/lab-solutions/software-tools/worklist-generator.html

Table 1: Example worklist. *Please note: Table header must be written **exactly** as shown.*

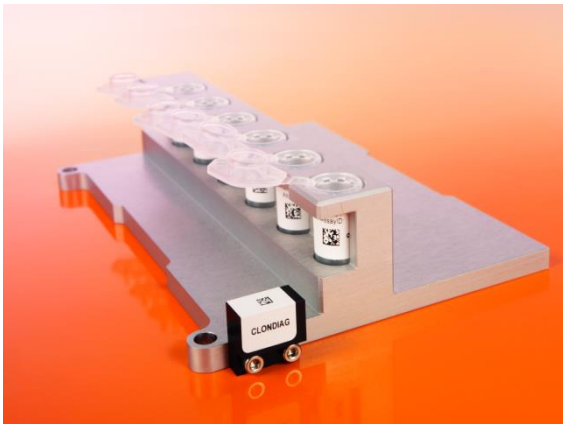
position	sampleID	assayID
1	DD_018_UI_007807_MK_L50_010415	050103
2	DD_038_UI_007961_L50_010415	050103
3	VLW-B2162_00-419_L50_010415	050103
4	102_VLW-81_L50_010415	050103
5	DD_EPEC E2348.69_L50_010415	050103
6	14050313_501_L50_010415	050103

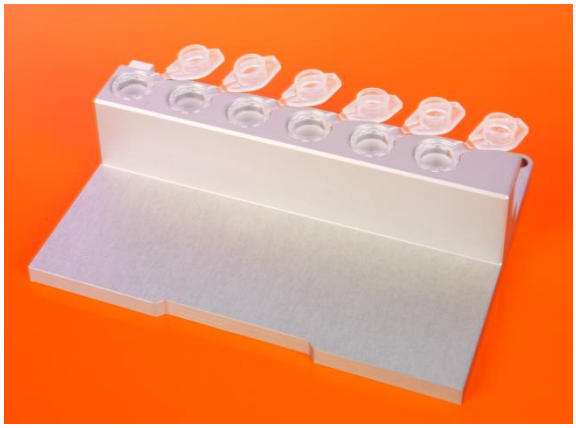
Data Acquisition in the ArrayMate Reader

- Insert your memory stick containing the worklist into any of the USB ports down to the right hand side of the ArrayMate.
- Press ; a folder selection dialogue will open.
- Select your worklist (path: “My Computer/Removable Disk”).
- Open your selected worklist by pressing **Enter** or **Open**.
- Press (your imported worklist opens in a separate window). Proofread. If the new window is empty, or if it was the wrong worklist, repeat the import.
- Press **OK**; the worklist window will close.

- Leave the memory stick in the ArrayMate if you intend to export **AMR-ve Genotyping** Test Reports afterwards (check the memory stick for computer viruses and malware using an appropriate program on a regular basis).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Carefully insert the appropriate metallic adapter/frame into the ArrayMate. Do not apply strong force. Assure proper fit, otherwise the images may be out of focus.
- After having inserted the adapter, carefully insert the Array Tubes into the adapter.

  ArrayTubes need to be open with tube-lid connections placed into appropriate notches:





- **Assure proper fit**, otherwise the images may be out of focus.
- Barcodes on ArrayTubes and holder must be clean.
- Press **Next** (at the bottom right on the screen; reader closes, analysis program starts, it takes about 2-10 min, depending on the number of ArrayTubes; the reader takes images and automatically analyses the data). The progress of the reading is indicated by the following symbols:

photographed: in analysis: ready:

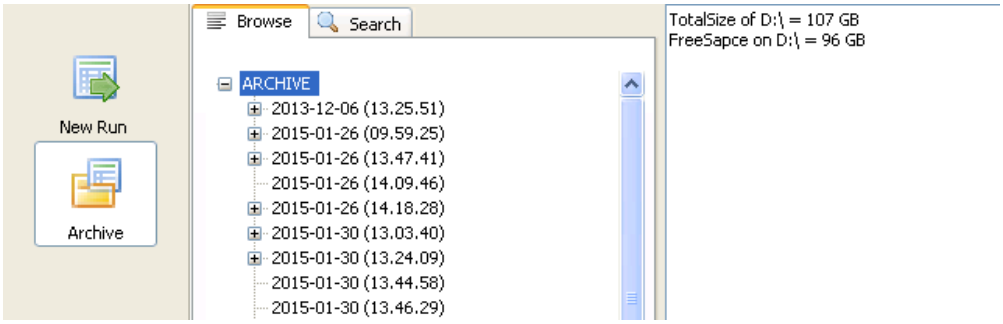
- The reader indicates the end of the entire process with an acoustic signal (beep).
- Press **Next** (at the bottom right on the screen; reader is opening).
- Remove the adapter with the ArrayTubes.
- Press **Next** (at the bottom right on the screen; reader is closing).

Results

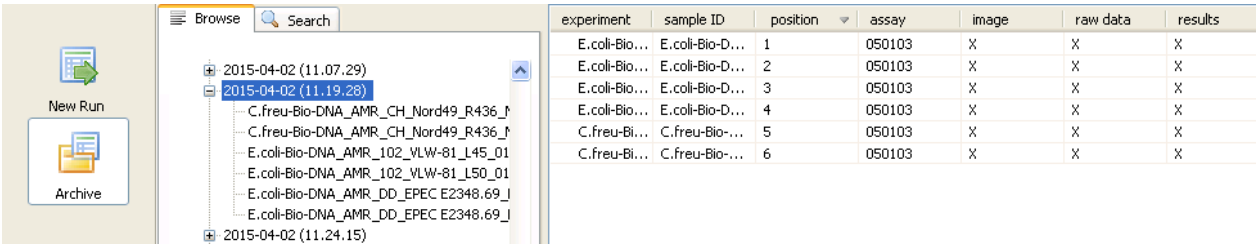
On the left-hand side of the screen you will see a list showing all runs stored on the ArrayMate´s hard disk. A run contains the results from all arrays analysed together within one frame. If this list is not displayed:

- Press **Archive** (left hand side) and activate the flag **Browse** (at the top left).
- The runs are organised like folders in **Windows Explorer**, and named **by default** according to the date of acquisition.

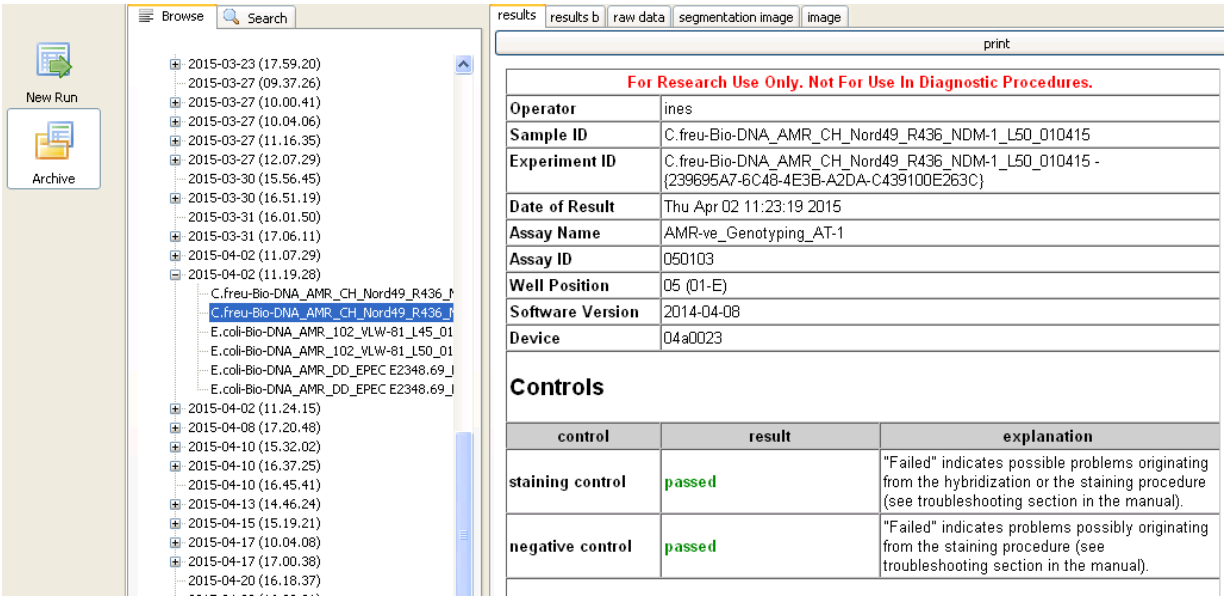
Example: there are several readings in the archive, by default they are named by date and time of day of creation (which you may have changed, see section “setup of the ArrayMate reader”):



If you click on the plus symbol left on the run name, the folder opens and you will see a list of the individual arrays ordered by Sample ID.

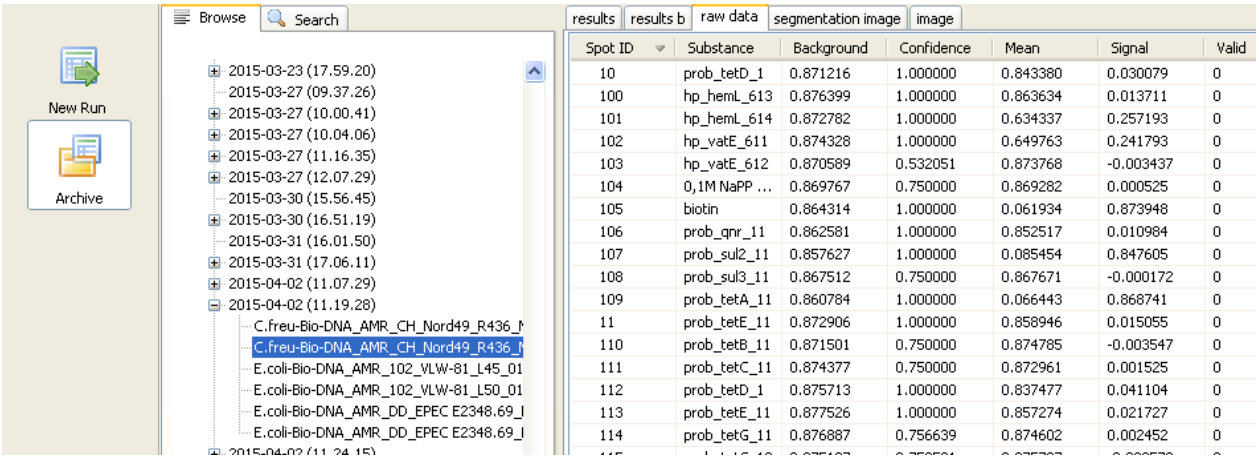


Activate the tab **results** (top left) and click onto the position of an individual experiment the report of this particular array will appear on the right side of the window:



*Please Note: the flag **results b** is not active with this assay.*

Activate the tab **raw data** (top left) and the raw signal results of this particular array will appear on the right side of the window:

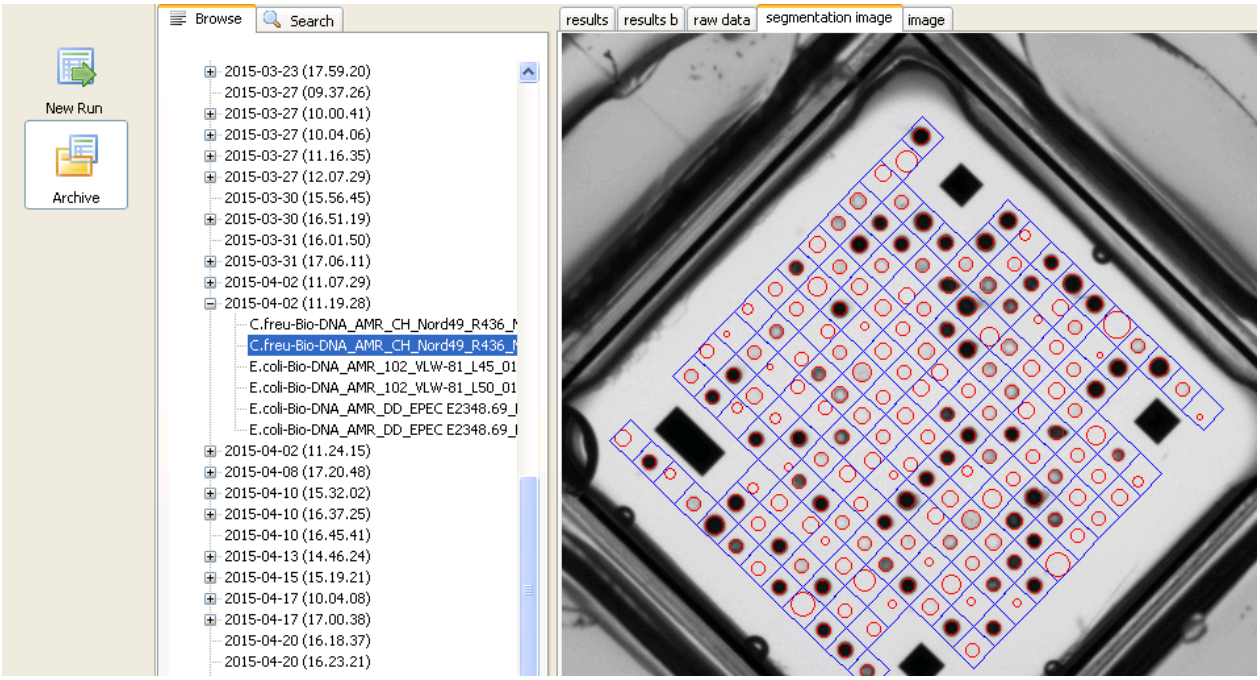


Spot ID	Substance	Background	Confidence	Mean	Signal	Valid
10	prob_tetD_1	0.871216	1.000000	0.843380	0.030079	0
100	hp_hemL_613	0.876399	1.000000	0.863634	0.013711	0
101	hp_hemL_614	0.872782	1.000000	0.634337	0.257193	0
102	hp_vatE_611	0.874328	1.000000	0.649763	0.241793	0
103	hp_vatE_612	0.870589	0.532051	0.873768	-0.003437	0
104	0,1M NaPP ...	0.869767	0.750000	0.869282	0.000525	0
105	biotin	0.864314	1.000000	0.061934	0.873948	0
106	prob_qnr_11	0.862581	1.000000	0.852517	0.010984	0
107	prob_sul2_11	0.857627	1.000000	0.085454	0.847605	0
108	prob_sul3_11	0.867512	0.750000	0.867671	-0.000172	0
109	prob_tetA_11	0.860784	1.000000	0.066443	0.868741	0
11	prob_tetE_11	0.872906	1.000000	0.858946	0.015055	0
110	prob_tetB_11	0.871501	0.750000	0.874785	-0.003547	0
111	prob_tetC_11	0.874377	0.750000	0.872961	0.001525	0
112	prob_tetD_1	0.875713	1.000000	0.837477	0.041104	0
113	prob_tetE_11	0.877526	1.000000	0.857274	0.021727	0
114	prob_tetG_11	0.876887	0.756639	0.874602	0.002452	0

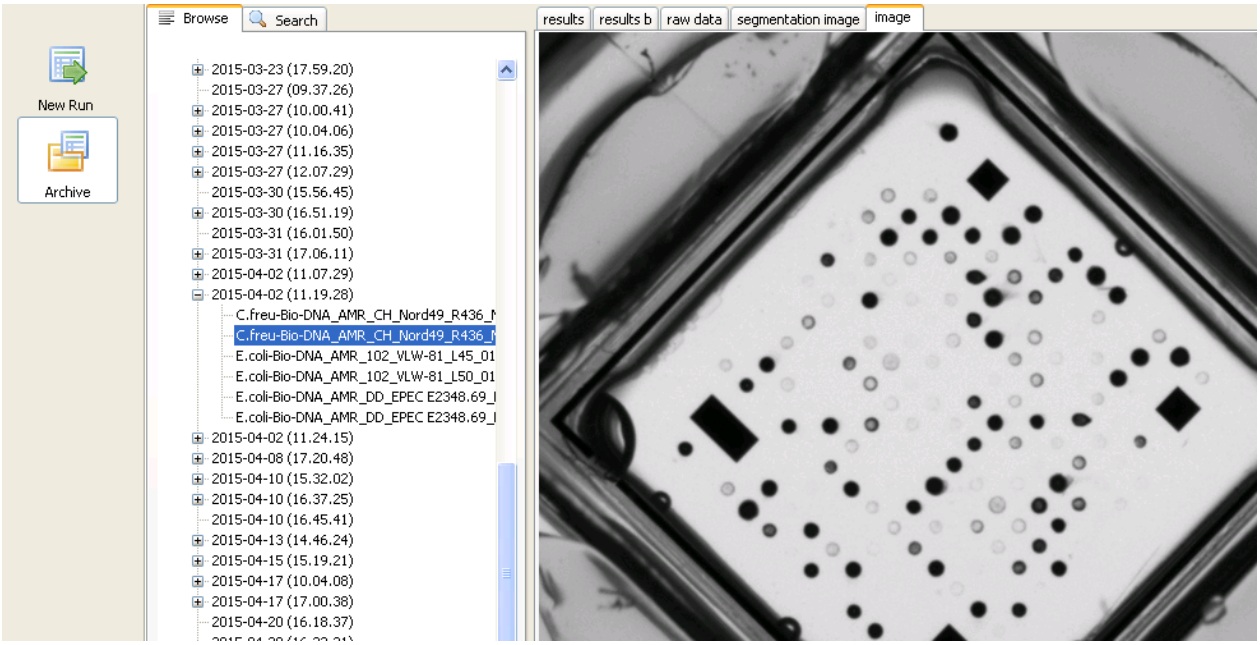
- Spot ID: numerical identifier of the spot on the array
- Substance: name of the DNA probe
- Confidence: an intrinsic estimate of spot confidence based on size and shape of that particular spot, where 1 = high confidence and 0 = no confidence
- Signal: spot signal intensity (grey scale value), where 1 = black and 0 = white
- Valid: 0 = valid; 1 = invalid (confidence below 0.75)
- Background: luminous intensity of the background, where 1 = maximum brightness and 0 = maximum darkness
- Mean: luminous intensity of the signal (spot), where 1 = maximum brightness and 0 = maximum darkness

Please note: The correlation between mean, background and signal is roughly: 1-mean/background, however, there are some correction factors that depend on the statistics of pixel distribution.

Activate the tab **segmentation image** and the analysed picture of this particular array will appear on the right side of the window:



Activate the tab **image** and the picture of this particular array will appear on the right side of the window:



Export of Test Results

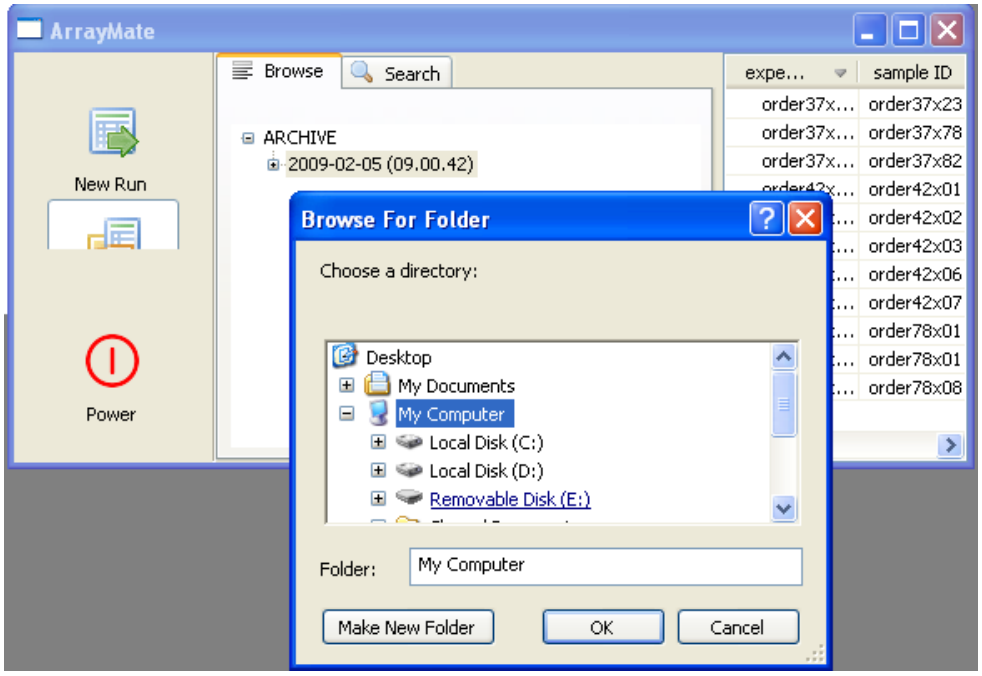
The generated result files in an html format will show information of all target genes. Possible invalid controls that might display in this report will be explained below (see Troubleshooting).

Other files that are generated and that can be exported include:

- A text file (*.txt) with the raw measurements (raw data),
- An image file (*.bmp) with the actual photo of the array,
- A second image file (*.png) in which the coordinate grid is superimposed and the recognised spots are circled (segmentation image), and
- A XML file (*.xml) that contains the same information like the html result sheets for future export into databases and for using the **Result Collector** tool.

*Please note: Only complete runs can be exported. The export of individual **E. coli Genotyping** Test Reports is not possible.*

- Right-click on the selected run (a menu appears with the option **Export Run Reports**).
- Right-click on **Export Run Reports** (a file browser opens).



- Click **My Computer**, then **Removable Disk** and choose the folder where to save or click **Make New Folder** (on the bottom, a new folder icon appears).
- Rename the new folder (e.g. with the experiment name or date).
- Click **Ok** (data are exported into the new folder on your memory stick).
- Do **NOT** remove the memory stick as long as the hourglass symbol is visible.
- Switch off the device by clicking **Power** (at the bottom left on the screen):
- Switch off the screen. There is no need to physically switch off the ArrayMate Reader.

TROUBLESHOOTING

In case of trouble always make sure that reagents are within the recommended shelf-life and stored under appropriate conditions.

Should you encounter a problem, we will always be happy to support you. Please e-mail to cct.home@clondiag.com and include a description of the problem as well as the array images (*.bmp files) in question.

Staining Control

A staining control is included to check whether possible problems originate from the hybridisation or the staining procedure. If the staining control has “Failed” proceed as follows:

Horseradish peroxidase conjugate may have degraded during storage. Add 1 µL mixture C3/C4 to 9 µL D1 (substrate). If the solution turns green within 3-5 seconds, the horseradish peroxidase still has sufficient enzymatic activity.

Enzymatic reaction is inhibited by carryover of buffer C1. Ensure proper washing with buffer C2 of the wells to remove all of buffer C1 prior to adding horseradish peroxidase conjugate.

If the staining control has “Passed”, refer to the following hints.

Image Quality

In case of poor image quality we recommend to re-check DNA quantity and quality first by loading leftover DNA on an agarose gel.

In order to determine whether any problems originated from the DNA preparation, you might perform an experiment with the Control Material CM^{EDL}. This is genomic DNA from *E. coli* EDL933 (GenBank accession number NC_002655.2). It is be provided free of charge upon request. If the control experiment yields a valid result and a correct identification of probes *gapA, ihfA, dnaE* and *hemL*, there was probably an issue with DNA preparation. If the control experiment also fails, an error affecting later steps or a degradation of reagents from later steps is likely.

See also Appendix 2 – Images for troubleshooting (p. 35 and 36).

DNA Quality

The amount of DNA is crucial because of the linear kinetics of amplification (see Introduction). DNA should be free of RNA, as free RNA reduces the efficiency of amplification and labelling by effectively removing primer from the reaction mix due to competitive hybridisation. A_{260} readings will cover RNA and other contaminants as well. Therefore pure DNA preparations without RNA contaminations are a prerequisite for proper DNA concentration measurement. RNAse treatment prior to A_{260} reading therefore is necessary.

DNA must be unfragmented, as fragmentation reduces the efficiency of amplification and labelling due to the distance between primer and probe binding sites. DNA should for this reason not be prepared by disrupting bacterial cells using bead beaters, ultrasonication or aggressive chemicals such as in alkaline lysis protocols. We made good experiences with the manual QIAGEN DNeasy kit and the automated device EZ1.

DNA must be free of any traces of ethanol, as ethanol strongly influences the amplification. It is possible to heat the sample prior to adding it to the labelling mix (5-10 minutes at 70°C). Some problems with samples from the Qiagen EZ1 device for example were resolved after heating the samples (see above).

Physical Damage to the Array

Scratching of the array surface with a pipette tip can lead to the damage of array spots that prohibits the acquisition of a valid signal. In this case the respective marker is not assigned as "negative", but instead the message "none" appears next to the marker name.

Ambiguous Results

Apart from a "positive" or "negative" result for the individual markers on the **AMR-ve Genotyping** Test Report, the result can also be "ambiguous".

In cases affecting markers for genes associated with resistance, no definitive answer with regard to this specific marker can be given. This can be caused by poor sample quality, poor signal quality and, especially in some resistance-associated genes by the presence of plasmids in low copy numbers.

Allelic variants of some markers differ only in single or few nucleotides. This can cause the effect that the actual allele yields a positive signal while other, mismatching probes give ambiguous rather than negative results.

ADDITIONAL INFORMATION

Warranty

Alere guarantees the performance as described in this manual. Usage of the Kit was successfully tested at ambient temperatures up to 37 °C. A guarantee is limited to ambient temperatures in the laboratory between 18 °C and 28 °C. Kit components comprise the ArrayTubes, the reagents for DNA labelling and for detection of labelled DNA products on the array, the ArrayMate reader and its software. In case one of these components fails within the expiry date due to other reason than misuse, contact Alere for replacement or refund. Terms and conditions apply.

If you have any problem or question, please contact the technical service.

Disclaimer

This system is for research use only.

We do not accept any liability for damages caused by misuse. Misuse comprises, especially but not exclusively, of a use of the system for the detection of resistance genes in order to predict (phenotypic) antibiotic resistances or susceptibilities for the guidance of an antibiotic chemotherapy.

Since resistance might be caused by genes or mutations not covered by this array or by hitherto unknown genes or mutations, any antibiotic chemotherapy MUST be guided by phenotypic susceptibility tests.

Furthermore, we do not accept any liability for damages caused by inappropriate use of the device as a personal computer, for instance related to the use of additional software, to network connections, or to a breach of privacy related to the storage of confidential information (such as names of patients) on its hard disk and/or to the use of external storage devices that might be contaminated with spyware.

Quality Control

Each batch is stringently tested with the use of standard DNA preparations for good performance and correctness of results.

List of Components for Separate Order

If required, these reagents may be ordered separately:

component	name	amount	cat#	storage
B1^{AMR}	Labelling Buffer	360 µL	245503000	2-8 °C
B2	Labelling Enzyme	20 µL	245104000	2-8 °C
C1	Hybridisation Buffer	30 ml	245105000	18-28 °C
C2	Washing Buffer 1	120 ml	245106000	18-28 °C
C3	HRP Conjugate 100x	200 µL	245107000	2-8 °C
C4	Conjugate Buffer	30 ml	245108000	18-28 °C
C5	Washing Buffer 2	120 ml	245109000	18-28 °C
D1	HRP Substrate	15 ml	245110000	2-8 °C
CM^{EDL}	Control Material *E. coli* EDL933 DNA	30 µL	on request	2-8 °C
ArrayTubes	AMR-ve05m	5 ATs	201009284	15-28 °C

For pricing please contact your local representative or our customer service, respectively.

Legal Manufacturer

Alere Technologies GmbH

Loebstedter Str. 103-105

07749 Jena, Germany

Contact

If you require any further information on this product please contact cct.home@clondiag.com

LITERATURE

1. Development of a miniaturised microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria. Batchelor M, Hopkins KL, Liebana E, Slickers P, Ehricht R, Mafura M, Aarestrup F, Mevius D, Clifton-Hadley FA, Woodward MJ, Davies RH, Threlfall EJ, Anjum MF; Int J Antimicrob Agents. 2008 Feb 1.
2. Identifying antimicrobial resistance genes of human clinical relevance within *Salmonella* isolated from food animals in Great Britain. Muna F. Anjum, Suman Choudhary, Victoria Morrison, Lucy C. Snow, Muriel Mafura, Peter Slickers, Ralf Ehricht and Martin J. Woodward; J. Antimicrob. Chemother. (2011)

For further literature please refer to:

http://alere-technologies.com/en/science-technologies/publications/

UPDATES & SOFTWARE

Notifications on database/software updates and freeware tools can be found at:

http://alere-technologies.com/en/products/lab-solutions/e-coli/e-coli-genotyping-kit.html

http://alere-technologies.com/en/products/lab-solutions/software-tools.html

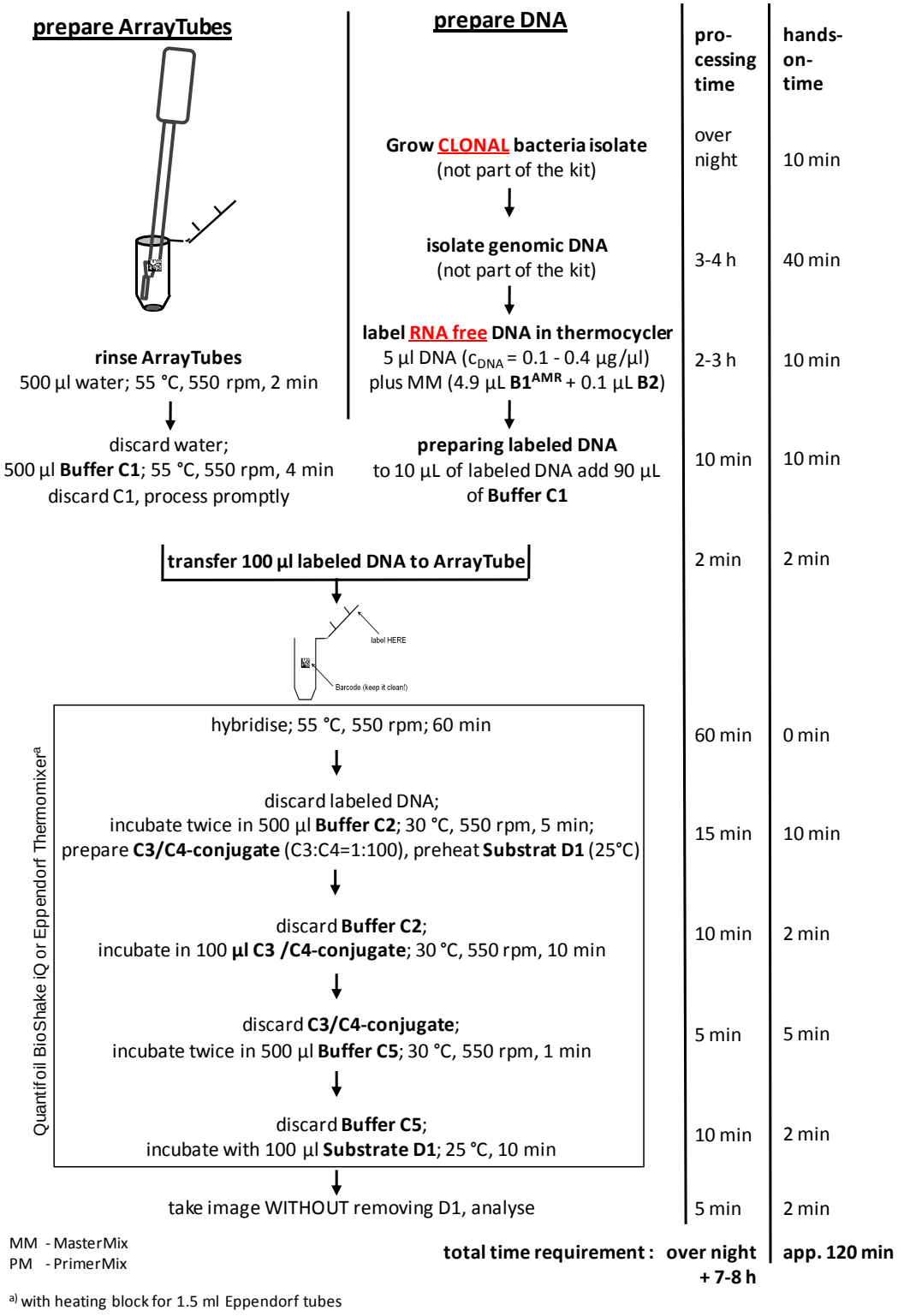
and/or http://alere-technologies.com/en/news.html

Currently available freeware programs are:

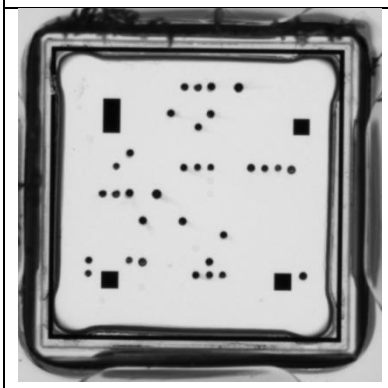
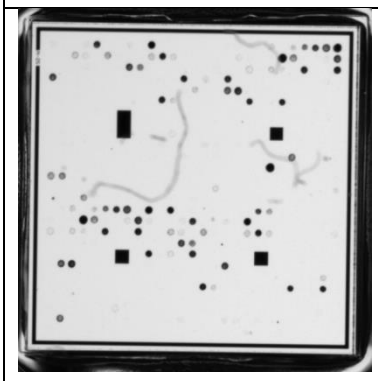
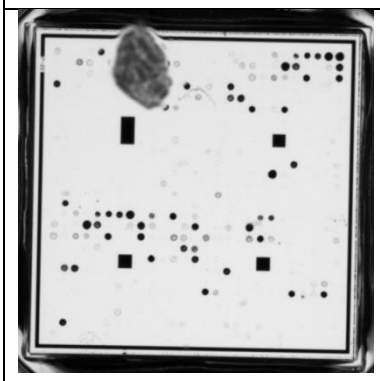
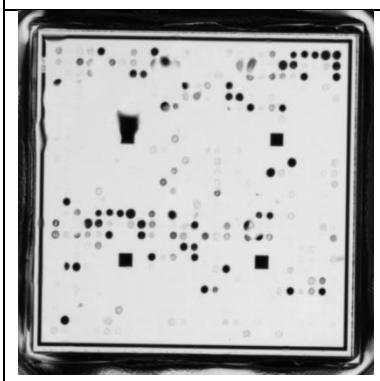
- Alere **Result Collector** for the conversion of multiple *result.xml files from the ArrayMate into spreadsheet tables. This should make it easier to compare isolates or to determine relative abundances of genes or strains etc.
- Alere **Worklist Generator** is a tool which helps you to create a well formatted worklist for the Arraymate.
- Alere **Report Generator** is a software tool to create reports using the assay software normally used and installed on the ArrayMate. It uses an image taken by the ArrayMate or a txt file (raw signal data file) and generates a report from the raw signal data.

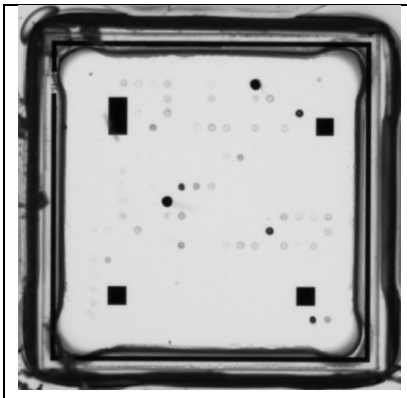
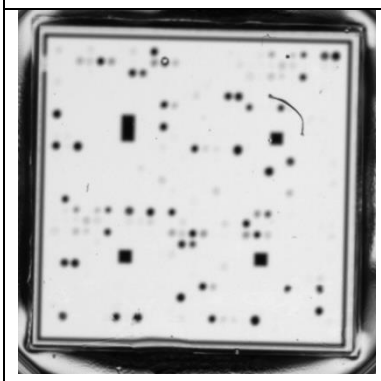
APPENDIX 1 - FLOW CHART

The figure on this page summarises the test procedure. However, please refer to the text section of this user guide at any step of the test protocol for further important details.


prepare ArrayTubes
prepare DNA
pro-cessing time
hands-on-time
Grow CLONAL bacteria isolate
(not part of the kit)
over night
10 min
isolate genomic DNA
(not part of the kit)
3-4 h
40 min
label RNA free DNA in thermocycler
5 µl DNA (cDNA = 0.1 - 0.4 µg/µl)
plus MM (4.9 µL B1AMR + 0.1 µL B2)
2-3 h
10 min
rinse ArrayTubes
500 µl water; 55 °C, 550 rpm, 2 min
discard water;
500 µl Buffer C1; 55 °C, 550 rpm, 4 min
discard C1, process promptly
preparing labeled DNA
to 10 µL of labeled DNA add 90 µL of Buffer C1
10 min
10 min
transfer 100 µl labeled DNA to ArrayTube
2 min
2 min
label HERE
Barcode (keep it clean!)
Quantifoil BioShake iQ or Eppendorf Thermomixer[a]
hybridise; 55 °C, 550 rpm; 60 min
60 min
0 min
discard labeled DNA;
incubate twice in 500 µl Buffer C2; 30 °C, 550 rpm, 5 min;
prepare C3/C4-conjugate (C3:C4=1:100), preheat Substrat D1 (25°C)
15 min
10 min
discard Buffer C2;
incubate in 100 µl C3 /C4-conjugate; 30 °C, 550 rpm, 10 min
10 min
2 min
discard C3/C4-conjugate;
incubate twice in 500 µl Buffer C5; 30 °C, 550 rpm, 1 min
5 min
5 min
discard Buffer C5;
incubate with 100 µl Substrate D1; 25 °C, 10 min
10 min
2 min
take image WITHOUT removing D1, analyse
5 min
2 min
MM - MasterMix
PM - PrimerMix
total time requirement :
over night + 7-8 h
app. 120 min
a) with heating block for 1.5 ml Eppendorf tubes

APPENDIX 2 – IMAGES FOR TROUBLESHOOTING

Image	Comment	Handling
	Valid experiment.	Valid results, no error messages.
	The bottom of the AT is contaminated with dust particles.	Please clean the bottom of the well, scan and process again.
	The microarray surface is contaminated with dust particles.	If the microarray surface is contaminated with particles, wash the microarray with double distilled water (pipetting water carefully up and down, remove), scan and process again.
	The bottom of the AT is contaminated with a liquid (e.g. buffer).	Please clean the bottom surface with a cleanroom wipe, scan and process again.

	Signal intensity is too low.	This could be due to low DNA concentration, fragmented DNA, ethanol trace contaminations in DNA sample or expired reagents. The experiment should be repeated with a new DNA preparation. If this also fails, try an experiment with EDL933 control DNA (CM, available on request).
	Chip was not in focus during image acquisition.	Repeat image acquisition after fitting the ArrayTube in the frame.

APPENDIX 3 - GENE LIST

AMR family	Gene	Probe	Synonym	Description[1,2]
acetyltransferase	vatE	hp_vatE_611	vatE-3; vatE-4; vatE-5; vatE-6; vatE-7; vatE-8; satG	acetyltransferase;streptogramin A acetyltransferase; associated with resistance to streptogramin A (AF242872.1)
acetyltransferase	vatE	hp_vatE_612	vatE-3; vatE-4; vatE-5; vatE-6; vatE-7; vatE-8; satG	acetyltransferase;streptogramin A acetyltransferase; associated with resistance to streptogramin A (AF242872.1)
aminoaminoglycoside resistance	aac	prob_aac3Ia_1	aacC1	3-N-aminoglycoside acetyltransferase; associated with resistance to astromicin; gentamicin; sisomicin (U90945.1)
aminoaminoglycoside resistance	aac	prob_aac3IVa_1	aacC4	3-N-aminoglycoside acetyltransferase; associated with resistance to apramycin; dibekacin; gentamicin; netilmicin; sisomicin; tobramycin (EU784152.1)
aminoaminoglycoside resistance	aac	prob_aac6Ib_1	aacA4	aminoglycoside 6'-N-acetyltransferase; associated with resistance to streptomycin, spectinomycin (M21682.1)
aminoaminoglycoside resistance	aadA	prob_aadA1_1	aadA1	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (EU704128.1)
aminoaminoglycoside resistance	aadA	prob_aadA2_1	aadA2a; aadA2b; aadA2c; aadA3; aadA8	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (EU704128.1)
aminoaminoglycoside resistance	aadA	prob_aadA4_1	aadA5	aminoglycoside adenyltransferase; associated with resistance to streptomycin, spectinomycin (EU704128.1)
aminoaminoglycoside resistance	ant2	prob_ant2Ia_1	aadB	aminoglycoside (2'') adenylyltransferase; associated with resistance to dibekacin; gentamicin; kanamycin; sisomicin; tobramycin (L06418.4)
aminoaminoglycoside resistance	strA	prob_strA_611	aph3	aminoglycoside-3''-phosphotransferase (locus A); associated with resistance to streptomycin (EF090911.1)
aminoaminoglycoside resistance	strB	prob_strB_611	aph6	aminoglycoside-6''-phosphotransferase; associated with resistance to streptomycin (EF090911.1)
beta lactam resistance	acc	prob_acc2_11	blaACC-1; ACC-1a4; ACC-2; ACC-4	class C beta-lactamase (EF554600.1)
beta lactam resistance	acc	prob_acc1_11	blaACC-1; ACC-1a4; ACC-2; ACC-4	class C beta-lactamase (EF554600.1)
beta lactam resistance	act1	prob_act1_11	blaACT-1; ACT-2; ACT-3; MIR-8; Mir2; Mir4	class C beta-lactamase (U58495.2)

beta lactam resistance	cmy	prob_cmy_11		consensus sequences to blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35, blaCMY-CFE1, blaCMY-CFE2 (Citrobacter spp.), blaCMY-Cmur (Citrobacter murliniae), blaCMY-Cwer (Citrobacter werkmanii), blaCMY-Cyou (Citrobacter youngae), blaCMY-HG3, blaCMY-HG4
beta lactam resistance	cmy	hp_blaCMY_611		consensus sequence to blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35, blaCMY-CFE1, blaCMY-CFE2 (Citrobacter spp.), blaCMY-Cmur (Citrobacter murliniae), blaCMY-Cwer (Citrobacter werkmanii), blaCMY-Cyou (Citrobacter youngae), blaCMY-HG3, blaCMY-HG4
beta lactam resistance	cmy	hp_blaCMY_612		consensus sequence to blaCMY-13, blaCMY-2, blaCMY-24, blaCMY-35, blaCMY-CFE1, blaCMY-CFE2 (Citrobacter spp.), blaCMY-Cmur (Citrobacter murliniae), blaCMY-Cwer (Citrobacter werkmanii), blaCMY-Cyou (Citrobacter youngae), blaCMY-HG3, blaCMY-HG4
beta lactam resistance	ctx	prob_ctxM1_11	ctxM15; ctxM28; ctxM29; ctxM3; ctxM32; ctxM33; men-1	extended spectrum beta lactamase, class A beta-lactamase (X92506.1), including ctxM15 (HQ202266.1)
beta lactam resistance	ctx	prob_ctxM1_12	ctxM15; ctxM28; ctxM29; ctxM3; ctxM32; ctxM33; men-1	extended spectrum beta lactamase, class A beta-lactamase (X92506.1), including ctxM15 (HQ202266.1)
beta lactam resistance	ctx	prob_ctxM9_11	ctxM13; ctxM14; ctxM16; ctxM17; ctxM18; ctxM21; ctxM24; ctxM27; ctxM51; ctxM9a; ctxM9b; KLUY-2; KLUY-3; KLUY-4	class A beta-lactamase (AF174129.3)
beta lactam resistance	ctx	prob_ctxM2_11	ctxM20; ctxM3; ctxM35; ctxM5; ctxM6	extended-spectrum beta-lactamase CTX-M2, class A beta-lactamase (AM040709.1)
beta lactam resistance	ctx	prob_ctxM26_11	ctxM25; ctxM39; ctxM41	extended spectrum beta lactamase, class A beta-lactamase (AF518567.2)
beta lactam resistance	ctx	prob_ctxM8_11	blaKLUG-1; ctxM40; ctxM63	extended spectrum beta lactamase, class A beta-lactamase (AY750914.2)
beta lactam resistance	dha	prob_dha1_1	DHA-1; DHA-2	class C beta-lactamase (EF406115.1)
beta lactam resistance	fox	prob_fox_11	FOX-5; FOX-7	consensus sequence for blaFOX genes (AJ703795.1)
beta lactam resistance	len	prob_len1_11	blaLEN	class A beta-lactamase (AY743416.1)
beta lactam resistance	fox	prob_mox_1pm	blaFOX	consensus sequence for blaFOX genes (AJ703795.1)
beta lactam resistance	mox	prob_mox_1mm	blaMOX	consensus sequence to all blaMOX genes
beta lactam resistance	mox	hp_blaMOX-CMY9_611	ampC; blaCMY-10; blaCMY-9; MOX-4	class C beta-lactamase, extended-spectrum beta-lactamase precursor, associated with resistance to cephalosporins (AF381617.1)

beta lactam resistance	mox	hp_blaMOX-CMY9_612	ampC; blaCMY-10; blaCMY-9; MOX-4	class C beta-lactamase, extended-spectrum beta-lactamase precursor, associated with resistance to cephalosporins (AF381617.1)
beta lactam resistance	mox	hp_blaMOX-CMY9_613	ampC; blaCMY-10; blaCMY-9; MOX-4	class C beta-lactamase, extended-spectrum beta-lactamase precursor, associated with resistance to cephalosporins (AF381617.1)
beta lactam resistance	oxa	prob_oxa1_21	blaOXA-1; blaOXA-30; blaOXA-31; blaOXA-33	beta-lactamclass D beta-lactamase OXA-1 (AY458016.1)
beta lactam resistance	oxa	prob_oxa2_11	blaOXA-2; blaOXA-3; blaOXA-15; blaOXA-20; blaOXA-21, blaOXA-22; blaOXA-32; blaOXA-34; blaOXA-36	beta-lactamase OXA-2; oxacillinase (U63835.1)
beta lactam resistance	oxa	prob_oxa7_11	blaOXA-35	class D beta-lactamase OXA-7 (AY866525.1)
beta lactam resistance	oxa	prob_oxa9_11	blaOXA9	beta-lactamase OXA-9 (M55547.1)
beta lactam resistance	per	prob_per2_1		class A beta-lactamase PER-2; extended-spectrum beta-lactamase (X93314.1)
beta lactam resistance	pse	prob_pse1_1pm	blaCARB-2; PSE-1	carbenicillinase (Z18955.1)
beta lactam resistance	pse	prob_pse1_1mm	blaCARB-2; PSE-1	carbenicillinase (Z18955.1)
beta lactam resistance	shv	prob_shv1_11	blaSHV; PIT-2	class A beta-lactamase - consensus sequence for blaSHV genes, including extended-spectrum beta-lactamases (EF035566.1)
beta lactam resistance	tem	prob_tem1_1	blaTEM	class A beta-lactamase - consensus sequence for blaTEM genes, including extended-spectrum beta-lactamases (Y12694.1)
chloramphenicol resistance	cat	prob_catA1_11		chloramphenicol acetyltransferase (group A) (V00622.1)
chloramphenicol resistance	cat	prob_catIII_1		chloramphenicol acetyltransferase (type III) (AJ249249.1)
chloramphenicol resistance	cat	prob_catB3_11	catB4	chloramphenicol acetyltransferase (group B) (AJ009818.1)
chloramphenicol resistance	cat	prob_catB8_12		chloramphenicol acetyltransferase (AF227506.1)
chloramphenicol resistance	cml	prob_cmlA1_11	cmlA cmlA1 cmlA4 cmlA5 cmlA6 cmlA7	chloramphenicol transporter (EF113389.1)
chloramphenicol resistance	flo	prob_floR_11		florfenicol export protein (AF252855.1)
erythromycin resistance	ereA	hp_ereA_611	ereA2	type I erythromycin resistance (AY183453.1)
erythromycin resistance	ereA	hp_ereA_612	ereA2	type I erythromycin resistance (AY183453.1)
erythromycin resistance	ereB	hp_ereB_611	ereB	type II erythromycin resistance (AB207867.1)
erythromycin resistance	ereB	hp_ereB_612	ereB	type II erythromycin resistance (AB207867.1)
family marker	gapA	prob_gapA_611		glyceraldehyde 3-phosphate dehydrogenase A (marker for Enterobacteriaceae)

famly marker	ihfA	prob_ihfA_611		integration host factor subunit alpha (marker for Enterobacteriaceae)
genus marker	dnaE	hp_dnaE_611		DNA polymerase III subunit alpha (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	dnaE	hp_dnaE_612		DNA polymerase III subunit alpha (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	dnaE	hp_dnaE_613		DNA polymerase III subunit alpha (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	hemL	hp_hemL_611	gsa; popC	glutamate-1-semialdehyde aminotransferase (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	hemL	hp_hemL_612	gsa; popC	glutamate-1-semialdehyde aminotransferase (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	hemL	hp_hemL_613	gsa; popC	glutamate-1-semialdehyde aminotransferase (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
genus marker	hemL	hp_hemL_614	gsa; popC	glutamate-1-semialdehyde aminotransferase (Species marker for Salmonella spec., Escherichia spec. and Shigella spec.)
integrases	int	prob_intI1_1		class 1 integrase (AY260546.3)
integrases	int	prob_intI2_11		class 2 integrase (AY183453.1)
macrolide	ermB	hp_ermB_611		rRNA adenine N-6-methyltransferase, lincosamide and streptogramin B resistance protein (AB089505.1)
macrolide	ermB	hp_ermB_612		rRNA adenine N-6-methyltransferase, lincosamide and streptogramin B resistance protein (AB089505.1)
quinolione resistance	qnr	prob_qnr_11	qnrA1	quinolone or fluoroquinolone resistance protein (AY931018.1)
quinolione resistance	qnr	prob_qnrB_11	qnrB2; qnrB3; qnrB4; qnrB5; qnrB6	quinolone or fluoroquinolone resistance protein (AB281054.1)
quinolione resistance	qnr	prob_qnrB_12	qnrB2; qnrB3; qnrB4; qnrB5; qnrB6	quinolone or fluoroquinolone resistance protein (AB281054.1)
quinolione resistance	qnr	prob_qnrS_11		quinolone or fluoroquinolone resistance protein (AM234722.1)
quinolione resistance	qnr	prob_qnr_12	qnrA1	quinolone or fluoroquinolone resistance protein (AY931018.1)
sulphonamide resistance	sul	prob_sul2_11		dihydropteroate synthetase type 2 (DQ464881.1)
sulphonamide resistance	sul	prob_sul3_11		dihydropteroate synthetase type 3 (AJ459418.2)
sulphonamide	sul	prob_sul1_11		dihydropteroate synthetase type 1 (AJ698325.1)

resistance				
tetracycline resistance	tet	prob_tetA_11	tetA2; tetA3	tetracycline resistance protein A, tetracycline efflux protein (CP000971.1)
tetracycline resistance	tet	prob_tetB_11		tetracycline resistance protein A, class B (V00611.1)
tetracycline resistance	tet	prob_tetC_11		tetracycline resistance protein A, class C (EU751612.1)
tetracycline resistance	tet	prob_tetD_1		tetracycline resistance protein A, class D (X65876.1)
tetracycline resistance	tet	prob_tetE_11		tetracycline resistance protein A, class E (L06940.1)
tetracycline resistance	tet	prob_tetG_11	tetA(G)	tetracycline resistance protein A, class G (AF261825.2)
tetracycline resistance	tet	prob_tetG_12	tetA(G)	tetracycline resistance protein A, class G (AF261825.2)
trimethoprim resistance	dfr	prob_dfrA1_21	dhfrI	dihydrofolate reductase type I (AJ884723.1)
trimethoprim resistance	dfr	prob_dfrA1_22	dhfrI	dihydrofolate reductase type I (AJ884723.1)
trimethoprim resistance	dfr	prob_dfrA7_11	dfrA9; dhfrIX; dhfrVII	dihydrofolate reductase type 7 (AB161450.1)
trimethoprim resistance	dfr	prob_dfrA7_12	dfrA9; dhfrIX; dhfrVII	dihydrofolate reductase type 7 (AM237806.1)
trimethoprim resistance	dfr	prob_dfr12_11	dhfrXII	dihydrofolate reductase type 12 (AB154407.1)
trimethoprim resistance	dfr	prob_dfr13_11	dfrA21	dihydrofolate reductase type A13; dihydrofolate reductase type A21 (Z50802.3)
trimethoprim resistance	dfr	prob_dfrA14_21	dhfrIb	dihydrofolate reductase type 14 (AJ313522.1)
trimethoprim resistance	dfr	prob_dfrA15_1		dihydrofolate reductase type 15 (Z83311.1)
trimethoprim resistance	dfr	prob_dfrA17_11		dihydrofolate reductase type 17 (AF169041.1)
trimethoprim resistance	dfr	prob_dfrA19_1		dihydrofolate reductase type 19 (AJ310778.1)
trimethoprim resistance	dfr	prob_dfrV_21	dfrA5; dhfrV	dihydrofolate reductase type 5 (AB188269.1)

1) http://ardb.cbcb.umd.edu/index.html
2) http://www.uniprot.org/